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The Internal Secretion of the Pancreas

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IT is a pleasure to participate in this commemoration by the Academy of Medicine, Toronto, of the discovery of insulin. Many may not know that the first paper on insulin presented in Toronto, outside the University, was to this Academy.

The International Diabetes Federation and scientific and lay organizations in a number of countries have celebrated the fortieth Anniversary of the discovery of insulin and I have been invited to represent the University of Toronto on some of these occasions.

Fred Banting and I published four papers together, that is, as sole authors. I propose to confine my remarks, almost exclusively, to excerpts from them.

The first paper was entitled "The Internal Secretion of the Pancreas", and was presented on November 14, 1921, before the Physiological Journal Club of the University of Toronto (Fig. 1).

UNIVERSITY OF TORONTO

PHYSIOLOGICAL JOURNAL CLUB

Nov. 14th – 4 o'clock – Room 17

Speakers – Dr. Banting
Mr. Best

Subject – Pancreatic Diabetes

Fig. 1.—The notice of the meeting where the original paper on insulin was presented.

The original manuscript is interesting from many points of view. Some of the handwriting is Fred Banting's, some is my own, and some is that of the young lady who was then my fiancée and is now my wife; some is in a handwriting that cannot be definitely identified. This is, in my opinion, the most interesting memento of the discovery of insulin, apart from our actual notebooks which are preserved in the University of Toronto. I am hopeful that these and other memorabilia will find a home in the museum in the bridge between the Banting and the Best Institutes.

It has always seemed to me that one of the most interesting statements in our original paper was the following: "In the course of our experiments we have administered over 75 doses of extract from degenerated pancreatic tissue to ten different diabetic animals. Since the extract has always produced a reduction in the percentage of sugar of the blood and of the sugar excreted in the urine, we feel justified in stating that this extract contains the internal secretion of the pancreas."

This first paper[1] was sent for publication to the *Journal of Laboratory and Clinical Medicine* directly after it was presented in November at the Physiological Journal Club, and it appeared in the February number of volume 7 (1922). Our conclusions at that time, i.e. November 1921, were as follows:

"Intravenous injection of extract from dog's pancreas, removed from seven to ten weeks after ligation of the ducts, invariably exercises a reducing influence upon the percentage sugar of the blood and the amount of sugar excreted in the urine. Rectal injections are not effective.

"The extent and duration of the reduction varies directly with the amount of extract injected.

"Extract made with 0.1% acid is effectual in lowering the blood sugar. Extract prepared in neutral saline and kept in cold storage retains its potency for at least seven days.

"Boiled extract has no effect on the reduction of blood sugar. Pancreatic juice destroys the active principle of the extract.

"The presence of extract enables a diabetic animal to retain a much greater percentage of injected sugar than it would otherwise.

"That the reducing action is not a dilution phenomenon is indicated by the following facts: (1) hemoglobin estimations before and after administration of extract are identical; (2) injections of large quantities of saline do not affect the blood sugar; (3) similar quantities of extracts of other tissues do not cause a reduction of blood sugar."

The last paper[2] of which Banting and I were the sole authors was published in the *University of Toronto Medical Journal* in 1923. I propose to quote freely from that communication, which was entitled "The Discovery and Preparation of Insulin". After a concise (300-word) review of previous work we wrote:

"In 1921 authorities were agreed that all attempts to demonstrate the internal secretion of the pancreas had met with either negative or inconclusive results.

"The hypothesis upon which our experiments were founded was that some constituent of the external secretion of the pancreas was antagonistic to the hypothetical internal secretion for which Professor Shaffer of the University of Edinburgh had suggested the name 'Insuline'.* The hypothesis was presented to Professor Macleod of this University and he was able to provide advice and facilities for putting it to the test. Work was commenced in the Department of Physiology on May 16th, 1921. It was originally supposed that only

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*De Meyer, in 1909, was actually the first to suggest this name for the hypothetical internal secretion.

some six or eight weeks would be necessary to test the hypothesis.

"In order to eliminate the external secretion of the pancreas it was proposed to take advantage of the above mentioned fact that the acinous cells degenerate after the ligation of the pancreatic ducts. Our first work was, therefore, to ligate the pancreatic ducts of a number of normal dogs. The animals recovered quickly from the operation and none of them showed any symptoms or signs of diabetes. In the interval required to secure degeneration of the pancreas, we obtained records on totally depancreatized dogs which we contemplated using as test objects. The extreme heat of the summer of 1921 made it very difficult for us to keep the diabetic animals in reasonably good condition and we were not in a position to test any material until late in July.

"On July 27th we decided to make our first test. We anaesthetized a dog whose pancreatic ducts had been ligated some ten weeks previously and removed the degenerated pancreas. The gland was smaller than normal and very firm in consistency. It was immediately sliced into a chilled mortar containing Ringer's solution and the mixture was frozen. After several hours the frozen material was allowed to thaw slowly and the tissue was finely macerated with sand and a pestle. The liquid was then strained off and filtered through paper. This filtrate was the original pancreatic extract used in our experiments. The material was administered by intravenous injection to a diabetic dog. The material exerted a slightly favourable effect upon the blood sugar and sugar excretion of the animal. This experiment was not at all conclusive but extremely encouraging to us, and we made haste to repeat it. Subsequent experiments were very successful and we consistently observed that the administration of this extract of degenerated pancreas resulted in marked lowering of blood sugar, diminution of sugar excretion, and phenomenal improvement in the general condition of completely diabetic dogs. The administration of a single large dose of this material often changed a listless semi-comatose diabetic dog into a bright, active animal. . .

"Active extracts were prepared from the pancreas of foetal calves, taken at a stage of development before the pancreatic ferments had appeared. Potent extracts from normal adult ox pancreas were also secured. To obtain this latter material we extracted finely divided ox pancreas with acidulated alcohol. We consider this a very important experiment since all large scale methods for the preparation of insulin today have the acid alcohol extraction as the first step in the process. It was shown that a completely diabetic dog could be kept alive and healthy for at least 10 weeks by the daily administration of this whole gland extract.

"About the middle of December, 1921, Professor J. B. Collip was invited to participate in the biochemical side of the investigation, and was provided facilities by Professor J. G. Fitzgerald and Professor R. D. Defries of the Connaught Antitoxin Laboratories. A little later Mr. E. C. Noble, Drs. John Hepburn and J. K. Latchford began work on insulin under the direction of Professor Macleod in the Department of Physiology. It was ascertained that the administration of insulin enabled a completely diabetic dog to store large amounts of glycogen in its liver. It will be remembered that diabetic animals are unable to store greater than 2% of glycogen in the liver in the absence of insulin even when very large quantities of sugar are administered. It was found that the respiratory quotient of diabetic dogs could be definitely raised by the administration of carbohydrate and insulin. This experiment proved conclusively that insulin caused the burning of carbohydrate in the diabetic organism. [As better methods have been evolved this conclusion has been confirmed and extended.] The extracts used in the first experiments in which glycogen was found in the livers of completely depancreatized dogs and in some of the respiratory quotient experiments, were prepared from adult ox pancreas by our original method. This group also recognized the deleterious effects of overdoses of insulin and discovered that sugar was a very efficient antidote. The effects of insulin upon various forms of experimental hyperglycaemia were studied, particularly by Mr. E. C. Noble. Drs. Hepburn and Latchford showed that the presence of insulin resulted in the increased consumption of sugar by the perfused mammalian heart.

"The first insulin administered to diabetic patients in the Toronto General Hospital was prepared from normal ox pancreas by the original method. This material exerted a definite effect upon the blood sugar and sugar excretion of a diabetic patient. Certain objectionable effects, however, were observed, which were undoubtedly due to the large percentage of protein which this extract contained. [An interesting statement in view of the fact that insulin turned out to be a protein.]

"Professor Collip, with the knowledge of the properties of the material which we had obtained and with additional information which he secured by very extensive work, was able, in a very short time, to purify the original extract so that much of the objectionable inert material was eliminated. The results of the short study of the clinical effects of this material were reported by Drs. W. R. Campbell and A. A. Fletcher of the Toronto General Hospital, in collaboration with Professor Collip and ourselves.

"Insulin was originally prepared from the ox pancreas, as stated above, by the extraction of fresh glands with faintly acid alcohol. The concentration of alcohol in our original experiments varied from 40 to 60 per cent. The alcoholic solution of pancreas was filtered and the filtrate concentrated by evaporation of the alcohol and water in a warm air current. Lipoid material was removed

by extracting the residue with toluene or ether. . . The active material contained in this extract was practically insoluble in 95 per cent alcohol. Professor Collip used acid alcohol as the initial extractive but raised the alcohol concentration to about 80 per cent. By this means certain inert objectionable materials were removed. The inert materials were filtered off and the insulin precipitated from the alcoholic solution by raising the concentration of alcohol to approximately 92 per cent. The precipitated material was dissolved in distilled water. Certain difficulties were encountered when it was attempted to apply this method to larger scale production and various modifications were introduced by the workers in the Connaught Laboratories in the spring of 1922. It was found that the final product obtained by these methods was not sufficiently pure for prolonged clinical use, and efforts were made to secure a better product. The benzoic acid method of Moloney and Findlay, which depends upon the fact that insulin is absorbed from watery solution by benzoic acid, was successfully used in the Connaught Laboratories for several months. Professor Shaffer of Washington University, St. Louis, and his collaborators, Somogyi and Doisy, introduced a method of purification which is known as the isoelectric process.* This method depends upon the fact that if a watery solution of insulin is adjusted to approximately pH 5 a precipitate settles out which contains much of the potent material and relatively few impurities. Dudley has found that insulin is precipitated from watery solutions by picric acid and he has made use of this fact to devise a very ingenious method for the purification of the active material. Mr. D. A. Scott, and one of us (C.H.B.), who are responsible for the preparation of insulin in the Insulin Division of the Connaught Laboratories, have tested all the available methods and have appropriated certain details from many of these. Several new procedures which we believe are advantageous have also been introduced".

The third of the four papers on insulin was entitled "Pancreatic Extracts". This was published in the May (1922) number of the *Journal of Laboratory and Clinical Medicine*.[3] The essential features of this paper are adequately presented in our fourth[4] insulin paper, entitled, as was the first one, "The Internal Secretion of the Pancreas". It is the one that was read before the Toronto Academy of Medicine on February 7, 1922. It began with my presentation of a series of lantern slides to illustrate the points that we had reported in our first presentation, i.e. to the Physiological Journal Club, three months earlier. Fred Banting then read, from a prepared manuscript, an account of our more recent findings. I shall quote from parts of that paper:

"The results so far reported occupied our time until the middle of November, 1921, when a new era was introduced by the discovery that the foetal calf pancreas of under five months' development did not contain pancreatic juice but did contain internal secretion.

"Laguesse found that the Islets of Langerhans are comparatively more plentiful in the foetus and newborn than in the adult animal. On November 16th* the idea presented itself that by making an extract of the pancreas of foetal calves, we might be able to obtain large quantities of the internal secretion without the destroying influence of pancreatic juice. This was done and, to our great satisfaction, on the injection of such an extract, the blood sugar of a diabetic dog was reduced from 0.30% to normal and the urine became sugar-free. This was repeated both in the same dog and in other depancreatized dogs with a similar result.

"Carlson found that pregnant bitches depancreatized near term did not develop glycosuria until the pups were born. Allan was unable to confirm this result. Ibraham was unable to find proteolytic enzymes in the pancreas of the foetus of under four months' development.

"This finding gave us access to large quantities of potent extract and abolished the delay and expense of obtaining the extract by ligating the pancreatic ducts of the dog and waiting for degeneration. Furthermore, it offered strong evidence that the active principle is universal in the Animal Kingdom. (We have since tried the bovine extracts on dogs, rabbits, and the human, and the results confirm this view).

"Foetal calf extract was prepared by macerating the glands in [acid] Ringer's solution and filtering until a clear solution was obtained. To get an idea of the potency of the extract so obtained, we placed 50 gms. of tissue in 250 c.c. of saline, macerated and filtered; 15 c.c. of this solution were then diluted in 250 c.c. with saline. A 15 c.c. dose of this solution reduced the percentage of blood sugar in a 10 kilogram dog from 0.40% to 0.15% in three hours.

"Up to this time the extract had been given intravenously. We found that this extract when given subcutaneously gave a slower and more prolonged, but not less marked, fall in percentage of blood sugar.

"In the endeavour to secure a sterile extract, we next tried the effect of preservatives. We found that 0.7% tricresol, which is double the strength used in preserving diphtheria anti-toxin, did not interfere with the active principle. It was also found that the extract could be Berkfeldted, but much of its potency was lost in this procedure.

"Alcohol was then tried in the place of saline as an abstractive, and it was found that the active

*Mr. George Walden of Eli Lilly and Company independently discovered this phenomenon and applied it to the large scale preparation of insulin.

*This was two days after our first presentation (on November 14) and I can remember vividly that we interrupted our revision of the manuscript for publication to discuss, with considerable excitement and anticipation, the new idea.

principle was soluble. . . Consequently, we macerated a whole beef pancreas immediately after the death of the animal in 95% alcohol. After allowing it to stand 12 hours, the liquid was squeezed out and filtered until clear. This solution was then evaporated to dryness in a warm air current. The resin-like residue was re-dissolved in saline and injected subcutaneously into a diabetic dog. The percentage of blood sugar fell from 0.35 to 0.08 in three hours and the urine became sugar-free. This was repeated with similar results. We had thus obtained from the whole gland an extract of the active principle which, when washed with toluol, gave a brownish powder, which could be kept sterile, which was soluble in saline, and which in minute doses (50 mgms.) gave a pronounced effect on the sugar of the blood.

"At this stage of the investigation we secured the services of Dr. Collip, Professor of Biochemistry from the University of Alberta, Edmonton, on a year's leave of absence. He has worked intensively and has now obtained a very potent, soluble, more nearly protein-free extract which is being tested clinically.

"Previous to this we had anaesthetized and connected a diabetic dog to a blood pressure recorder. It was found that the protein-containing extract had a temporary but marked depressor effect. Samples of blood were taken every half-hour during the experiment and it was found incidentally that the percentage of blood sugar fell but slightly following the injection of a known, potent extract. This fact may, in part, account for the failures of some observers to obtain results.

"The question now arose as to why the extract acted but slightly under anaesthesia. It seemed reasonable to suspect that the glycogenic function of the liver was in some way involved as glycogen is not built up during anaesthesia. We cannot state definitely, but some of our results would lead us to believe that the presence of the internal secretion of the pancreas is necessary in order that the liver build up glycogen from sugar.

"Dog 19 was our first attempt to keep a depancreatized animal alive with artificially administered internal secretion of the pancreas. This dog lived 19 days. Our next attempt was on Dog 27; this dog lived 21 days; death was caused by an anaphylactic-like reaction following an injection of calf extract. When Dog 27 died, we were using Dog 33 as a trial dog for the effects of the various forms of extract. This dog had been given almost every variety of extract we had prepared and by every method of administration. We had from time to time given therapeutic doses of extract, and at the end of 20 days, the dog was in excellent condition. We then decided to use her for a longevity experiment. Extract was given twice per day, later once per day. The animal was fed on lean meat, milk and dog biscuits. A slight gain in weight was noted. At the end of 70 days the dog was becoming thinner and weaker but was still able to walk and wag her tail. The animal was then chloroformed and an autopsy performed by Dr. Robinson. No islet tissue whatever was found. . . Under favourable conditions, a totally diabetic dog may be kept alive for a considerable length of time if the internal secretion of the pancreas is administered.

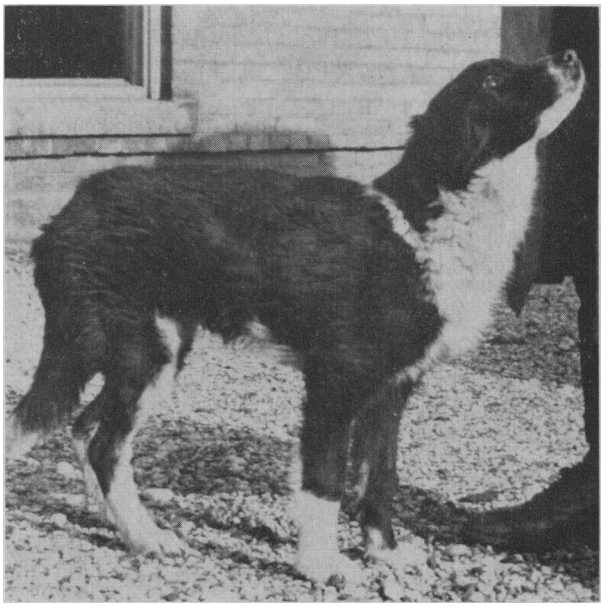

Fig. 2.—The first insulin-treated depancreatized dog to live for a prolonged period.

"In order to prove that this substance is only found in the pancreas, extracts of spleen, liver, thymus, muscle and thyroid were tested. Thyroid extract alone gave a slight fall in blood sugar.

"At the present time Mr. Best and Dr. Hepburn are conducting experiments on the respiratory quotient. Their results, though not sufficient in number as yet to report, strongly indicate that carbohydrate is not burned in the tissue in totally diabetic dogs, but is burned after pancreatic extracts are given. For example, the Respiratory Quotients before extract is administered range from 0.68 to 0.74, after extract from 0.85 to 0.94.

"At the present stage of investigation, the results we have to offer are purely experimental and afford no basis for assuming that the extract could be used in curing diabetics. Before the therapeutic value of the extract can be determined it would be not only necessary to conduct many more laboratory experiments, but also to investigate the effects of the extract in a diabetic clinic. For this purpose, we have been fortunate in securing the co-operation of Professor Duncan Graham and his associates, Drs. Walter R. Campbell and Almon A. Fletcher. . .

"We cannot prepare a sufficient amount of the extract at present to take care of more than the needs of the Medical Clinic and of the research on animals. As soon as a thorough clinical test has been made and details regarding dose, method of administration, and indications for use, are worked out, we hope that we may have the opportunity of presenting a further report to the Academy."

Canad. Med. Ass. J.
Nov. 17, 1962, vol. 87

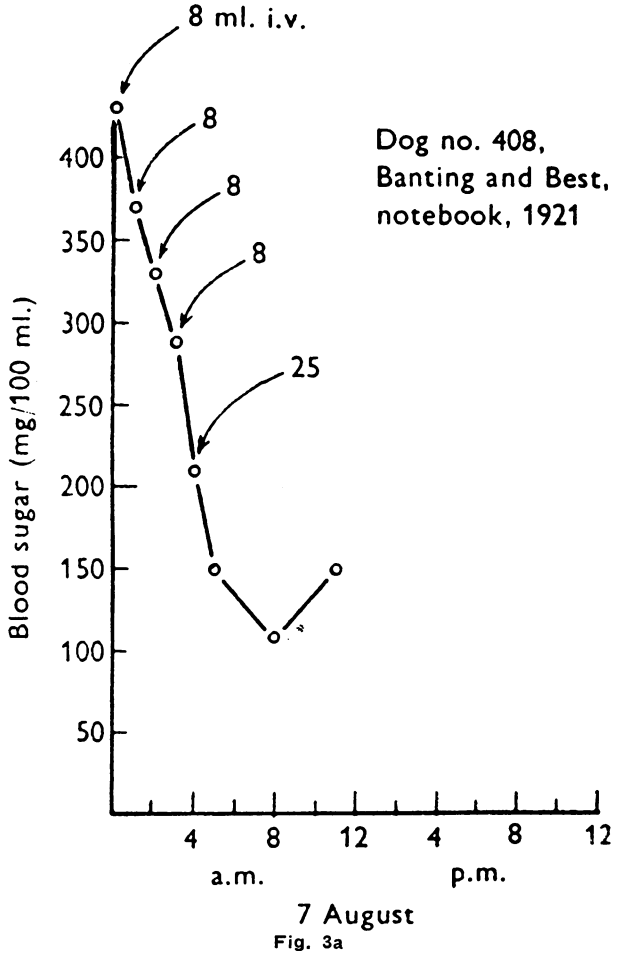

Fig. 3a

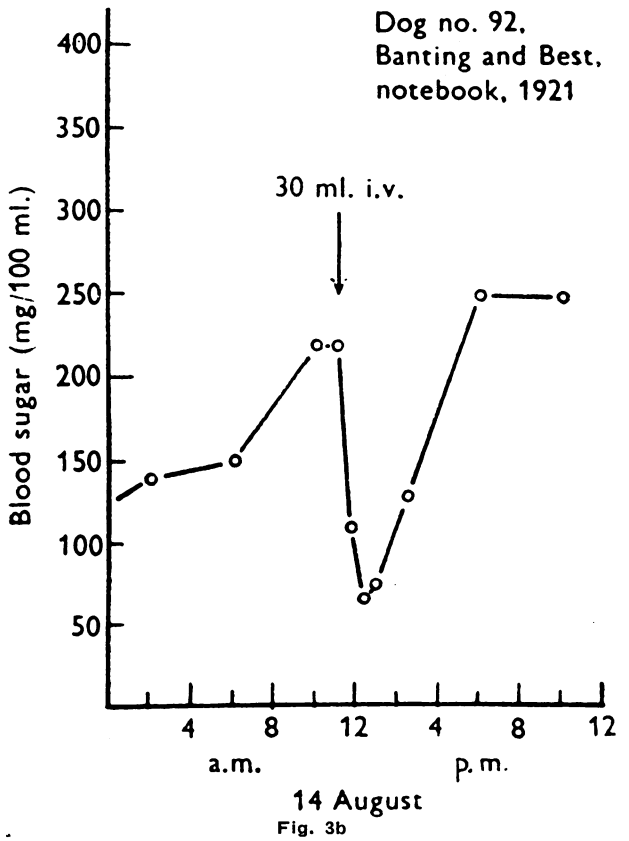

Fig. 3b

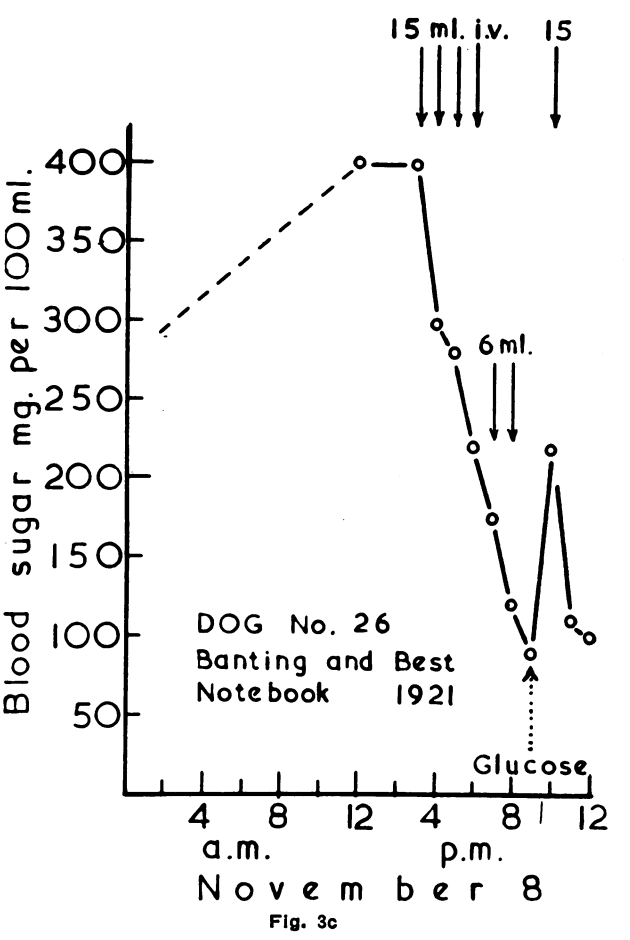

Fig. 3c

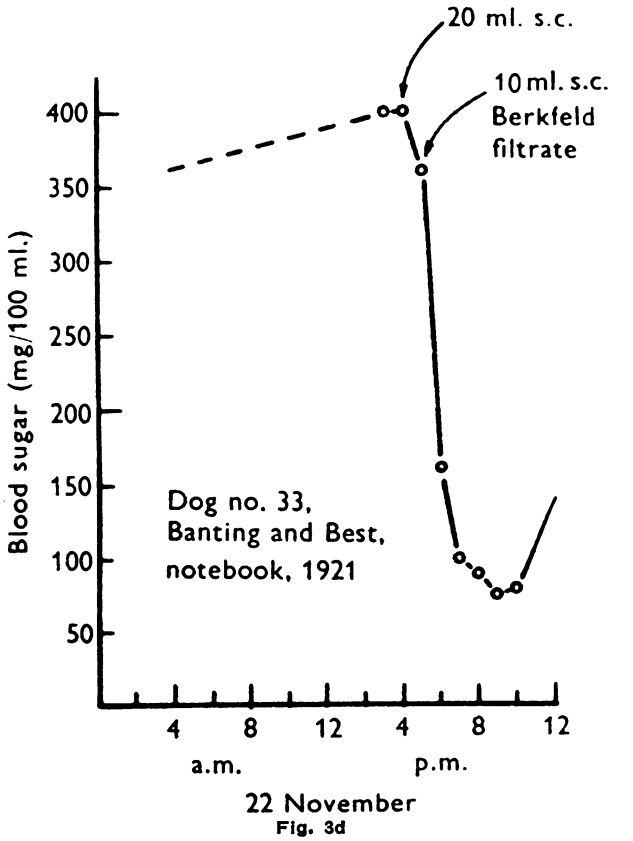

Fig. 3d

Fig. 3.—Effects of injections of extracts of degenerated pancreas in depancreatized dog. a. The first dramatic lowering of blood sugar by insulin (August 7, 1921). b. The first production of hypoglycemia by insulin (August 14, 1921). c. The first alleviation by sugar of the signs of hypoglycemia due to a rapid and extensive lowering of blood sugar (November 8, 1921). d. The rapid lowering of blood sugar by a very potent extract of fetal calf pancreas (November 22, 1921).

At the beginning of this last paper, every detail of the presentation of which I remember with great clarity, because it was the first scientific communication made outside of the University of Toronto in which I had participated, there is a reference to lantern slides to illustrate a number of points. I shall not confine myself exactly to those points but, instead, have reproduced in Fig. 3 certain of the findings which Fred Banting and I obtained during the first nine months of our collaboration.

In conclusion, I will quote from an Appreciation of Frederick Grant Banting,[5] which I wrote in 1941. He was, of course, the most wonderful man I have ever known and the greatest scientist which Canada has produced. This appreciation of him was published in the *Proceedings of the Royal Society of London,* and I will quote only the following:

"The Second World War found Banting occupying the leading place in Canadian medical science and the esteem of the Canadian people. He was almost immediately made the Head of the Central Medical Research Committee of the National Research Council of Canada. Banting was ready for this new responsibility because from the time of Munich, he had made up his own mind that war was inevitable and he had taken steps to initiate aviation medical research in his own department. This is not the place to describe in detail his tireless efforts from one coast of Canada to the other, to stimulate research workers to undertake problems of national importance. He was able, through his popularity with men in other fields and his unceasing efforts, to secure funds and to organize the work of a large group of scientists who devoted themselves to war problems.

"When it became necessary in 1940 to effect a liaison with medical workers in Great Britain, Banting insisted on going himself. He carried valuable information in both directions, and on his return to Canada he threw himself again into research and organization. No risks were too great for him and he undertook many hazardous investigations in which he himself was the subject. When a second trip to England became necessary he welcomed the opportunity, but he had a premonition that the end was near. He died in a remote spot in Newfoundland on the 21st February, 1941, when the bomber in which he was travelling crashed in a forced landing. Banting died as he had lived—in the service of his country and of humanity. He is survived by his wife, the former Henrietta Ball whom he married in 1939, and by one son of a previous marriage, William Robertson Banting.

"The Banting Institute, the Banting Foundation, and the Banting Memorial Lectureship, have been created in the University of Toronto as tangible tributes to him, but his name will live for ever in the hearts of successive generations of diabetics and in the minds of young investigators who will be stimulated by his brilliant and fearless career."

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PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

DIABETES MELLITUS

It used to be taught that diabetes is not a common disease. [However] Each year seems to prove the increasing commonness of this serious pathologic state. . . By far the greatest number of cases of diabetes mellitus are those which are due to what may be called errors of metabolism.

There are, it is true, other causes: nervous strain, the excessive use of starches, an hereditary tendency to glycosuria, the so-called gouty diathesis, over-functioning of the thyroid, as in exophthalmic goitre—all seem to play a part in the aetiology of diabetes mellitus.

Accepting, then, these various factors as causative elements, it is not difficult to understand why the disease is becoming yearly more common. The nervous stress and strain of American life, the astounding lack of care in eating—not only as to the quantity of food but as to the kind, and the rapidity with which it is eaten—these all too common and well-known dietetic and hygienic sins are quite sufficient to explain the greater number of diabetics which statistics and experience, together, prove to exist.

Before leaving the subject of aetiology, it seems worth while to mention a possible, but generally overlooked, cause of the disease. I refer to the enormously increased consumption of glucose or "corn syrup" during the last half-decade. Practically all other sugars used as foods need elaboration in the body before they reach the form of grape sugar (glucose), which is necessary for their assimilation. In the case of commercial glucose, which forms the bulk of most of the syrups now on the market, the substance is already in what may be called the end-product stage. Is it not reasonable to suppose, therefore, that a person who, for some of the reasons given, has a tendency to glycosuria, may find his organism completely overwhelmed by the ingestion of the already elaborated glucose when taken in the form of corn syrup? In other words, glucose, when taken into the system, can pass directly into the blood stream, instead of taking the lower and more circuitous route through the liver, that the other sugars require. How large an aetiologic factor the increased consumption of commercial glucose may be, I cannot state, but I am convinced from much clinical experience that quite a large percentage of these cases is added to the increasing list.—A. J. Hodgson: *Canad. Med. Ass. J.*, 2: 874, 1912.